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## Release of the Biological Control Agent *Puccinia jaceae* var. *solstitialis* for Management of Yellow Starthistle at Fort Hunter Liggett, CA

by Judy F. Shearer and Andrew M. Hamblin

**PURPOSE:** This technical note describes the results of a field study conducted to evaluate the effectiveness of the rust fungus, *Puccinia jaceae* var. *solstitialis*, as a biological control agent for management of yellow starthistle, *Centaurea solstitialis* L., at Fort Hunter Liggett, an Army training installation in California (Figure 1).



Figure 1. A yellow starthistle infestation at Fort Hunter Liggett, CA. Note the spines on the seed heads.

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**BACKGROUND:** Fort Hunter Liggett is a United States Army fort in southern Monterey County, California, about 250 miles (402 km) north of Los Angeles and 150 miles (241 km) south of San Francisco. The fort is primarily used as a training facility, where activities such as field maneuvers and live fire exercises are performed. The fort is bordered on the north by Salinas Valley, on the east by the Santa Lucia Mountains, on the west by the Los Padres National Forest, and on the south by the Monterey and San Luis Obispo County line. The fort originally comprised 80,937 ha (800 km<sup>2</sup>), but even at its present size of 67,987 ha (668 km<sup>2</sup>), it is the largest United States Army Reserve command post.

Yellow starthistle is one of the most serious weed problems on Fort Hunter Liggett. The invasive plant was introduced into California in the 1870's but did not become a significant weed until the 1960's. Three factors contributed to its spread: extensive road building, increased suburban development, and expansion of the ranching industry (DiTomaso et al. 2006). Over the past 40 years, mainly through human activities, the noxious weed has spread into rangelands, native grasslands, orchards, vineyards, pastures, roadsides, and wasteland areas. Today the weed infests approximately 14 million acres in California alone (DiTomaso et al. 2006). As of 1999, yellow starthistle had infested 8,094 ha or 12 percent of Fort Hunter Liggett. Today, acreage estimates on the installation exceed 9,308 ha.

On military installations such as Fort Hunter Liggett, yellow starthistle can severely impact training exercises and can impair the use of equipment or clog air filters on vehicles. It can also cause severe injury to humans when the spines of the flower head are encountered. It is particularly problematic when yellow starthistle invades drop zones.

Yellow starthistle is a winter annual that can grow up to 2 m in height at maturity (DiTomaso 2005). Seed germination starts in the fall and can continue into winter and spring depending on moisture availability (Benefield et al. 2001). Following germination, the plant allocates resources initially to root growth and then to leaf expansion, stem development, and flower production (Sheley et al. 1993). Root growth is rapid and can extend beyond 3 ft in depth (DiTomaso et al. 2003). The plant forms a rosette by late winter and bolts in late spring or early summer. Bolting plants send up branched flowering stalks that produce yellow flower heads adorned with spines up to 5 cm long. Flowering begins in May and continues into October.

Various methods are in use to control yellow starthistle infestations. Mechanical control consists of hand pulling or hoeing small infestations, and tillage or mowing on larger acreages (DiTomaso et al. 2006). Cultural control techniques are used to manipulate the environment by burning, grazing, or revegetation efforts. Five insect species and a fungus have been officially released as biological control agents in California (DiTomaso et al. 2006). A sixth insect, the false peacock fly, was accidentally released in Oregon and is now found commonly throughout California on yellow starthistle (Balciunas and Villegas 1999). Unfortunately all of the insect species only attack the flower heads. Those causing the most damage are the hairy weevil and the false peacock fly (Woods et al. 2002; Pitcairn et al. 2003). While seed production may be reduced between 43 and 76 percent at some locations by the two insects, yellow starthistle infestations may produce over 100 million achenes per acre, providing sufficient seeds for the following growing season regardless of the impact of the agents (DiTomaso et al. 2006). Additional agents that attack other plant parts are needed. The Mediterranean rust disease, *Puccinia jaceae* var. *solstitialis*, was approved for release in California in 2003 (Woods et al. 2003;

Bruckart et al. 2005). The rust produces lesions on the rosette and stem leaves of yellow starthistle, inducing stress and reducing plant vigor. Several chemicals are available for yellow starthistle management, with clopyralid as the formulation Transline® being the most commonly used herbicide at Fort Hunter Liggett. An integrated weed management program utilizing all technologies has been implemented for yellow starthistle control at Fort Hunter Liggett since 1999.

*Puccinia jaceae* var. *solstitialis* is an autoecious macrocyclic rust having a life cycle with five spore stages on a single host plant (Agrios 2005). During a growing season, the rust produces multiple generations of urediniospores that become airborne and can infect other leaves and plants thus increasing the incidence, intensity, and spatial spread of the disease (Fisher et al. 2008). During plant senescence, many rust fungi produce survival spores (teliospores) that overwinter. The following spring when conditions are conducive to growth, the teliospores germinate to produce basidiospores that are thought to infect yellow starthistle seedlings to produce pycnia (Fisher et al. 2008). Pycnia have been observed on yellow starthistle plants in California where the rust was released the previous season (Fisher et al. 2006). Pycnia produce aecia, which produce urediniospores to complete the infection cycle of the rust (Agrios 2005; Savile 1970).

The first field release of *P. jaceae* var. *solstitialis* in the United States outside of containment was on July 9, 2003 in Napa County, California (Bruckart et al. 2005). Field infection was confirmed three weeks later by observing both urediniospores and teliospores; however, the organism did not naturally spread from the inoculation site due to high temperatures and low moisture availability, environmental constraints that inhibit fungal reproduction and infectivity (Bruckart et al. 2005). In 2005, a study was initiated at two locations in California with the goal of developing an optimum release strategy for the rust (Fisher et al. 2007). Plants in 1-m x 0.5-m plots were inoculated starting in January and ending in June at both locations. Although infection was noted at both sites following each inoculation the first year, reinfection occurred at only one site the next year. Reinfection was higher in plots inoculated early in the season (January, February, and March) than in those inoculated later in the season (May and June) (Fisher et al. 2007). The data also suggested that local climatic factors, particularly moisture availability, were likely to determine where the rust would be able to multiply and persist.

Between 2004 and 2006 the California Department of Food and Agriculture oversaw 176 field releases of *P. jaceae* var. *solstitialis* (Woods et al. 2007). The releases were spread over 41 counties encompassing the range of yellow starthistle in the state. In 2004 and 2005, approximately 90 percent of the sites had rust pustules on the leaves one to three months after inoculation (Woods et al. 2007). In 2006, the number was much lower due to environmental limitations (particularly moisture and temperature) on infectivity of the rust. Many of the 2006 releases were made at less than ideal times (early summer versus spring) and at less than ideal locations such as dry hillsides and urban areas. One year following release only 20 percent of all inoculated sites became reinfected. By years two and three, the reinfection percent dropped to 9.5 and 3 percent, respectively.

Since yellow starthistle has the potential to become a severe weed problem at other military installations, a research initiative was begun in the fall of 2007 at Fort Hunter Liggett to determine if the rust fungus could offer another management tool for the noxious weed. The goals of



the study were to: 1) monitor urediniospore infection over the yellow starthistle growing season, 2) monitor teliospore development, and 3) monitor reinfection over time.

**MATERIALS AND METHODS:** *Puccinia jaceae* var. *solstitialis* field isolate FDWSRU-84-71 was used for host specificity testing (Bruckart 1989; Bruckart et al. 1999) and was the isolate that was approved for release in California in 2003 (Bruckart et al. 2005). Urediniospores for the present study were propagated at the California Department of Food and Agriculture, Sacramento, CA, using methods described by Woods and Popescu (2004) and Woods et al. (2009).

In March 2008, 19 permanent 1-m × 1-m plots marked by wooden stakes were installed at Fort Hunter Liggett. Sixteen plots were located with different aspects on hillsides facing northeast and northwest to provide potentially different microclimates (Figure 2), plus three plots were placed in a low-lying area along a creek bed that might hold moisture longer. The inoculum for each plot was prepared by adding 100 mg of urediniospores to 200 ml of deionized water in a 250-ml beaker and adding 2-3 drops of Tween 20. The spore mixture was agitated for approximately 2 min to evenly disperse and wet the spores and then filtered through cheesecloth into a finger pump sprayer. The urediniospores were sprayed evenly over the plots to run off. Following application the plots were covered with 1-m<sup>2</sup> “dew tents” made of PVC pipe and black plastic to conserve moisture and promote infection of the rust. The tents remained in place overnight and were removed the following morning.



Figure 2. Overview of the site at Fort Hunter Liggett where 19 plots were set up to evaluate the biocontrol fungus, *Puccinia jaceae* var. *solstitialis*, March 2008.

Following the urediniospore application, the following measurements were taken: number of plants per plot, mean leaf length, and percent cover of yellow starthistle, grasses and forbs (Table 1). Also noted was the growth stage of the plants (i.e. seedling, rosette, bolting, flowering).

<b>Table 1. Yellow starthistle (YST) plants/plot, mean leaf length, percent cover of YST, forbs, and grasses in 19 1-m<sup>2</sup> plots at Fort Hunter Liggett, CA, March 2008.</b>					
<b>Plot #</b>	<b>YST Plants/plot</b>	<b>YST mean leaf length</b>	<b>% Cover YST</b>	<b>% Cover Forbs</b>	<b>% Cover Grasses</b>
1	57	11.8	10	5	5
2	61	9.6	10	10	10
3	89	15.0	40	10	5
4	310	13.1	70	5	5
5	71	13.5	30	5	30
6	38	12.0	5	0	70
7	32	12.7	5	5	30
8	31	8.8	10	10	20
9	265	17.0	60	20	20
10	183	15.2	15	10	10
11	47	16.0	20	5	10
12	43	16.5	15	20	20
13	63	18.1	15	30	30
14	50	16.1	20	5	5
15	55	16.1	10	5	70
16	109	18.9	50	5	20
17	157	12.7	25	25	20
18	121	17.7	40	30	5
19	125	17.1	35	5	5

Plants were scored as positive or negative for disease incidence two weeks, four weeks, and 10 weeks post inoculation in 2008. Incidence was determined by selecting a subset of plants from plots that had high numbers of yellow starthistle plants or looking at each plant in those plots with low numbers of plants. Ten plants in each plot were selected for disease severity measurements. Three of the most severely infected leaves on each plant were rated as follows: 1 (1-4 pustules/leaf); 2 (5-15 pustules/leaf); 3 (16+ pustules/leaf not uniformly distributed); 4 (light uniform pustules); 5 (heavy uniform pustules). The 10 plants in each plot were also used to determine if aspect was incidental in disease development. If disease was absent from the leaves, the plant received a score of zero; if disease was present on any of the three leaves, the plant received a score of one.

Plots were reevaluated one year following inoculation on two occasions to verify establishment of the rust in the field.

Analysis of variance (ANOVA) (Statistica Version 8, StatSoft, Tulsa, OK) was used for statistical treatment of data. Mean separations were accomplished using Tukey's Honest Significant Difference (HSD) test. Test of significance was conducted at  $P \leq 0.05$ .

**RESULTS AND DISCUSSION:** Most plots had a combination of yellow starthistle, grasses, and forbs (Table 1). Cover of yellow starthistle varied from 5 to 70 percent with as few as 31 plants per plot to as many as 310 plants per plot. In all plots, the leaves had expanded to the point that a relatively large surface area was available for spore deposition thus providing multiple sites for initial infection. Temperatures at the time of application reached approximately 18 °C and dropped to 0 °C overnight. Although the temperatures dropped to freezing the night following inoculation, the plots were protected with the “dew tents” and urediniospores have a relatively thick cell wall that would protect the contents from freezing.

At the time of inoculation of *P. jaceae* var. *solstitialis* in March 2008, plants were either still in the seedling stage or had formed rosettes. On the weekend following inoculation, Fort Hunter Liggett received approximately 1 in. of rain. Unfortunately no rainfall events were recorded the rest of the spring and summer months. Unusually high temperatures combined with the lack of rain produced conditions that were not conducive for good initial or secondary infection in the field.

Two weeks following inoculation at Fort Hunter Liggett a site visitation was made to evaluate infection rates in the 19 plots. Plants were carefully examined in each of the plots for disease symptoms (chlorotic lesions) that appear on the upper surface of infected leaves. Lesions are expected to appear two to four weeks following inoculation (Woods and Villegas 2006); however, no chlorotic spots were observed on yellow starthistle leaves in any of the plots. Dale Woods, the California Department of Food and Agriculture plant pathologist in charge of distributing the rust in California, was extremely surprised that not a single symptom was observed.<sup>1</sup>

Four weeks following inoculation, a second site visit was made to evaluate plant status and urediniospore infection rates. Plants in all but one of the plots were bolting with average plant height at 19.0 cm. None of the bolted plants had started to flower. Plants in 14 of the 19 plots had disease symptoms on the rosette leaves. In each case, disease pustules containing urediniospores were forming on the undersides of the leaves; however, percent infection was very low with few plants in most plots showing symptoms or signs (spores present) of infection (Table 2). The mean rate of infection for all the plots was 5.5 percent. This rate was eight-fold lower than infection rates recorded in 2004 at various locations in California where the mean rate of infection was 42.6 percent (Woods and Villegas 2005). Mean disease severity was also low (0.29) with only 51 leaves out of 570 examined showing some disease development. There were no significant differences in disease incidence ( $P = 0.088$ ) or severity ( $P = 0.051$ ) in relation to aspect or slope of the plots (northeast facing, northwest facing, or creek bed).

Ten weeks following inoculation, plants in all plots had bolted and were beginning to flower. As the plants bolt, the rosette leaves tend to turn brown and senesce. Although rust was still present on some rosette leaves that hadn't senesced, there was no evidence that it had infected stem leaves. The mean rate of infection was down to 1.9 percent, an approximate three-fold decrease from the previous evaluation (Table 2). The mean disease severity was also much reduced (0.008) with only 30 leaves out of 570 examined showing some disease development. It was interesting to note that one plot seemed to have considerably more diseased leaves than any other

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<sup>1</sup> Personal communication. 2008. Dale Woods, Plant Pathologist, California Department of Food and Agriculture.

plot. Compared to its nearest plot, both disease incidence and disease severity were significantly higher ( $P = 0.018$ ,  $P = 0.025$ , respectively). This was not true at the 4-week observation period when there were no significant differences between the two plots as to disease incidence or disease severity. In all likelihood the differences at the 10-week evaluation were due to higher leaf senescence in one plot than another. There were significant differences in disease incidence ( $P = 0.00005$ ) and severity ( $P = 0.00119$ ) in relation to aspect or slope of the plots. The northeast-facing plots had significantly greater incidence of disease than the northwest-facing plots but not those in the creek bed. Disease severity was significantly greater in northeast-facing plots than either northwest-facing or creek bed plots. This might be attributed to slightly greater shading from trees in the northeast-facing plots than the northwest or creek bed plots that were exposed to full sun most of the day.

**Table 2. Percent infection and mean disease severity in 19 yellow starthistle plots at Fort Hunter Liggett, CA, 4 and 6 weeks post inoculation with *Puccinia jaceae* var. *solstitialis*.**

Plot #	4 weeks post inoculation		10 weeks post inoculation	
	% infection	Mean disease severity	% infection	Mean disease severity
1	0	0	1.8	0.03
2	0	0	0	0
3	2	0.06	3.3	1.03
4	0	0	<0.0	0.03
5	6	0.50	1.4	0.03
6	18	1.37	15.8	1.03
7	22	0.06	3.1	0.10
8	23	1.13	9.7	0.13
9	0	0	0	0
10	2	0.23	0	0
11	9	0.23	0	0
12	5	0.13	0	0
13	5	0.37	0	0
14	6	0.40	0	0
15	4	0.20	0	0
16	3	0.16	0	0
17	1	0.53	1.0	0.03
18	0	0	0	0
19	2	0.06	1.0	0.03

There was no evidence in any of the plots that the rust formed teliospores. Typically as plants mature the uredia switch from producing urediniospores to producing teliospores (Agrios 2005). The teliospores do not germinate immediately nor are they able to directly infect yellow starthistle. They only serve as an overwintering stage of the fungus. The following spring when conditions are conducive to germination, they germinate sporogenically as basidiospores. The basidiospores are capable of infecting yellow starthistle. The extreme drought conditions in California in 2008 induced rapid dessication of the rosette leaves and did not allow ample time for the switch from urediniospore production to teliospore production, thus inhibiting development of the overwintering spore stage.



In 2009, two site visits were made to assess reinfection of yellow starthistle plants with the rust. The timing of the March visit was exactly one year after the initial inoculation in 2008. Plants in all plots were thoroughly examined but no chlorotic symptoms were observed on the leaves. Most of the plants were in the rosette stage with an average of six leaves per rosette (Figure 3a). Overall the plants appeared small and stunted compared to the previous year. Leaves were considerably shorter in length measuring an average of 4.5 cm compared to 14.6 cm the previous year.

No symptoms were observed on yellow starthistle during the second site visit in late May 2009. It was clearly evident that the plants were under severe drought stress. Many seedlings and rosettes were shriveled and dying (Figure 3b). Some of the plants had begun to bolt and flower but they were extremely stunted attaining only half the height of the previous year.

Fort Hunter Liggett does not have an official weather reporting station. From personal communication with the Integrated Training Area Management (ITAM) coordinator, the fort received about 1 in. of rainfall in December 2008, the first substantial amount since the previous March. The region received less than half the normal rainfall in 2008. The severe drought conditions continued into 2009 as evidenced by the stressed yellow starthistle plants in the field plots.

Most of the *P. jaceae* var. *solstitialis* field releases in California have been deemed unsuccessful (Woods et al. 2007) because the rust did not establish and spread. However, at one site in Sonoma County near San Francisco Bay, the rust established and spread east and somewhat north of the release site following the prevailing wind patterns (Woods et al. 2005).<sup>1</sup> The combination of cool temperatures with abundant moisture kept the yellow starthistle green and susceptible for an extended period of time during the spring and summer months, allowing the fungus to naturally spread. There is also some evidence that the rust might establish and spread at some locations in California's Central Valley (Fisher et al. 2008). Environmental conditions there resulted in high rates of infection both within a growing season and after a dormant season whereas in the coastal hills, infection decreased over time and rust was not observed the next season.

Environmental conditions at Fort Hunter Liggett are more similar to those of the California coastal hills than the Central Valley. High temperatures and lack of rainfall during the growing season are not conducive to the establishment and survival of the biocontrol fungus *P. jaceae* var. *solstitialis*. Bennett et al. (1991) determined that optimal conditions for infection occur at 15-20 °C and dew periods between 8 and 16 hr. In late winter/early spring optimal conditions may exist for a short period of time at Fort Hunter Liggett. This was the case in 2008 but not in 2009. In March and April of 2008 dew was present on the plants in the early morning; however, the temperatures exceeded 20 °C by the afternoon. In 2009 there was no dew on the plants in either March or May and in late May the temperatures were approaching 38 °C. Most of the annual rainfall the fort receives comes during the winter months, not during the seasons (spring and summer) that would be optimum for survival and spread of the agent. By May and June the rosette leaves are already becoming brown and senescing, offering little chance for the fungus to produce teliospores.

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<sup>1</sup> Personal communication. 2009. Dale Woods, Plant Pathologist, California Department of Food and Agriculture.

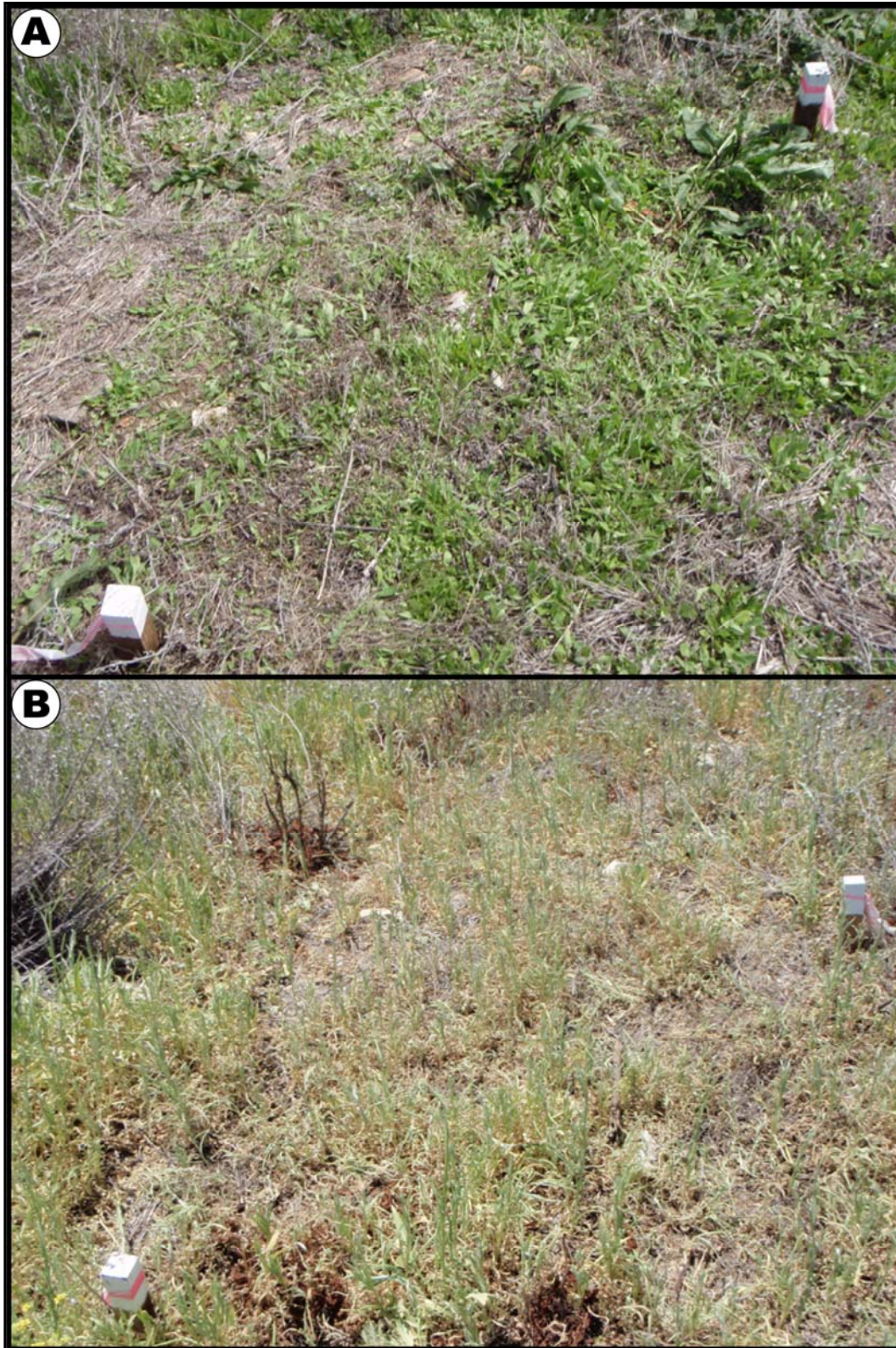


Figure 3. Yellow starthistle plot in March 2009 (a) and late May 2009 (b). Note how the plants are severely stressed in the bottom picture due to drought conditions at Fort Hunter Liggett.



Even though the rust does not appear to be a good biological control agent for yellow starthistle at Fort Hunter Liggett, it should not be ruled out as a tool at other installations where lower temperatures and moisture availability might be more suitable for infection and spread. If the temperature and dew period requirements could be satisfied, the fungus would probably do extremely well and stress the plant early in the growing season. Used in conjunction with seed-feeding insects, it is hoped that the guild of herbivores and pathogens will be sufficient to control yellow starthistle in parts of the western United States (DiTomaso et al. 2006). Installations in Oregon, Washington, and Idaho that have yellow starthistle infestations could probably greatly benefit from the use of the biological control agents. Compared to California, climatic conditions in those regions would definitely favor survival and spread of the rust pathogen.

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